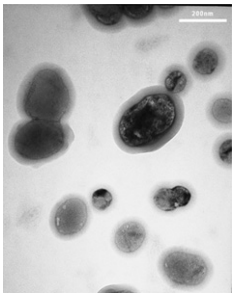
force for adsorption to the interface (Engin *et.al* 2010). By comparing the stability and surface tension of a series of alternative packing structures, we determine the internal structure of the monolayer, identify key residues for the stability, and make a relation between the structural organization and thermodynamics of the systems.

2092-Pos Board B78

Peptide Nanovesicles: Supramolecular Assembly of Branched Amphipathic Peptides

Sushanth Gudlur, Xiao Yao, Yasuaki Hiromasa, Takeo Iwamoto, John M. Tomich.

Research on non-lipid based carriers show promise in replacing lipid based delivery systems in delivering drugs. Peptide vesicles are one such class. We have designed and synthesized a set of (15, 19 and 24 residue), branched, amphipathic peptides that self-assemble into nanovesicles- 50 - 200 nm in diameter as determined by TEM and dynamic light scattering. Pairs of peptides with different lengths are mixed as helical monomers. After drying and redissolving in water, they undergo supramolecular assembly. CD studies show the assembled peptides adopt a predominantly beta-like conformation. Analytical ultracentrifugation data suggest that the peptide assemblies have a weighted average S value of 8. In addition to encapsulating and delivering both anionic and cationic fluorescent dyes, we have recently been able to deliver a 4.7 kbp plasmid (EGFP-N3), into MCF-7 cells grown on coverslips and observed expression of GFP. Peptide vesicles (Fig) are shown undergoing fusion, a property associated with lipid vesicles. We are currently exploring how to control size and stability by altering the ratios of the different chain lengths, as well as the ability to deliver plasmids of different sizes. These are potential drug delivery vehicles for targeted delivery.



2093-Pos Board B79

Tuning the Neurofilament Hydrogel Network - a Synchrotron X-Ray Scattering Study of Salt Dependent Response

Joanna Deek, Roy Beck, Cyrus R. Safinya.

Neurofilaments (NFs) are the cytoskeletal intermediate filament protein class expressed in neuronal cells and play a major a role in the maintenance and mechanical integrity of neuronal processes (i.e. the axon and dendrites). NFs assemble into flexible bottlebrushes from 3 different molecular weight subunits (NF-Low (NF-L), NF-Medium (NF-M), NF-High (NF-H)) with compositions that are specific for the axon and dendrites. The main variation in the subunits is the charge distribution of their unstructured C-termini sidearms. The sidearms are polyampholytes (i.e. containing both cationic and anionic amino acid residues), which enables interpenetration and interfilament attractions. We examine the strength and range of the electrostatic interfilament interactions by varying the salinity of the in vitro buffer. Reassembled (in vitro) binary system NF-hydrogels have revealed the different contributions of individual subunits to interfilament interactions and to network interfilament spacings [1-2]. This network tunability parallels with variable in vivo subunit expression in axons versus dendrites that results in variable network packing. We describe synchrotron x-ray scattering experiments that have allowed us to quantitatively study the changes in the microscopic structure of the NF gels as a function of salt and sidearm density. At high weight ratios of NF-M and NF-H, and as a function of increasing salt concentrations, NF gels exhibit an unexpectedly abrupt (most likely electrostatically driven) transition from a weakly oriented (nearly isotropic) low filament density gel with interfilament spacing d ~ 1000Å to a highly oriented liquid crystalline gel with high filament density and d ~ 500Å (NF-M) and ~ 700Å (NF-H).

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[1] R. Beck, J. Deek, J.B. Jones, C.R. Safinya. Nature Materials. 9, 40 (2010)
[2] J.B. Jones, C.R. Safinya. Biophysical Journal. 95, 823 (2008)

2094-Pos Board B80

Analysis of Bundle Formation in Biofilaments

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Bundles of biofilaments are ubiquitous in cells with functions ranging from force transmission to cellular protection and thus understanding the self-assembly of the biofilaments into bundles is crucial. The conformation of a bundle is dictated by a small number of mechanical and chemical parameters. In this work, given a set of parameters, our aim is to find the most favorable bundle conformation and the number of filaments within. The filaments are treated as discrete elastic rods. The final bundle structure is reached as a result of the competition between the elastic energy and favorable chemical interaction among individual filaments in the bundle. The results indicate that there is a variety of different size bundles with different conformations attainable, from dimers to hexagonal-closed-packed. We discuss our results along with case studies of important biofilaments.

2095-Pos Board B81

The Role of Bending Stiffness on the Rheology of Fibrin Networks

Huayin Wu, Louise Jawerth, David Weitz.

During the formation of a blood clot, fibrinogen is converted to fibrin which in turn polymerizes to form a biopolymer network whose mechanical properties are partially imparted to the clot itself. When such a network is deformed, it has an initial, low strain linear regime followed by a nonlinear, high strain regime. In the body, blood clots must function under a large range of stresses. For this reason, both the linear and nonlinear properties of a fibrin network are important for proper function. Previous work has studied the relation between calcium concentration and the linear modulus and fiber radius. Here we extend this work by also characterizing the effect of calcium concentration on the nonlinear modulus using rheology as well as on the hydrated structure using confocal microscopy. Unlike previous work, using this information, we can also directly test model predictions of the role of bending stiffness on both the linear and nonlinear moduli.

2096-Pos Board B82

Structural Basis of Bispecific Protein:Protein Interactions

Poorni Adikaram, Dorothy Beckett.

The ability of the *Escherichia coli* protein, BirA, to act as both an enzyme and a transcription repressor allows communication between metabolism and gene expression. BirA forms a hetero-dimer for the essential post-translational biotin addition to acetyl-CoA carboxylase and forms a homodimer to bind site-specifically to DNA and repress transcription initiation at the biotin biosynthetic operon. A single surface on BirA is used for both protein:protein interactions. However, the extent to which the structural information on this surface is functionally shared by the two interactions is not known. Multiple loops on the surface are located in both dimer interfaces. In this work alanine substitution variants of two loops, composed of residues 140-146 and 193-199, have been constructed and the proteins have been purified. Measurements of homo- and hetero-dimerization energetics of loop variant proteins using sedimentation equilibrium indicate that several residues in the two loops contribute to both interactions. Thus, structural information in both loops was important for evolution of bifunctionality in BirA.

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2097-Pos Board B83

Linkage Between SecA Dimerization and Ligand Binding

Andy J. Wowor, Dongmei Yu, Debra A. Kendall, James L. Cole.

The general secretion (Sec) pathway, found in bacteria, archaea, and eukaryotes, transports preproteins across membranes. The SecA protein mediates preprotein translocation through the SecYEG channel linked to ATP hydrolysis. Several studies suggest that SecA exists in a monomer-dimer equilibrium. Although self-association of SecA has been intensively studied, the oligomeric state of SecA, especially during preprotein transport, remains controversial. SecA dimerization is reported to be sensitive to salt concentration, temperature and ligand binding. We have characterized the energetics of SecA dimerization as a function of salt concentration, temperature and binding of a signal peptide using sedimentation velocity analytical ultracentrifugation. We employ fluorescence-detection to enhance sensitivity at low protein concentrations. SecA was labeled with Alexa Fluor 488 at the N-terminus. Labeling does not affect the dimer dissociation constant. Lower salt concentrations and higher temperatures greatly enhance dimerization. The dimer dissociation constants measured at 20°C range from 40 μM in 500 mM KCl to 14 nM in 100 mM KCl. Linkage analysis indicates that SecA dimerization is accompanied by the release of 5 ions. In addition, SecA dimerization is reduced upon binding of signal peptide, indicating that SecA oligomerization and ligand binding are thermodynamically linked.